## Application of Electrochemical Impedance Spectroscopy to Characterize Skin with Application to Transdermal Delivery of Therapeutic Drugs

M. A. Membrino, P. Shukla, and M. E. Orazem

Department of Chemical Engineering
University of Florida
Gainesville, Florida 32611-6005

Electrochemical Impedance Spectroscopy (EIS) has been used extensively to investigate the transport properties of skin. Motivation for the use of the EIS is provided in part by the ease of application of the technique coupled with the ability to collect a complete spectrum in a short time, e.g., within 5-10 minutes.

Despite the widespread application of Electrochemical Impedance Spectroscopy for transdermal drug delivery research, unambiguous interpretation of the data is not yet available. Rigorous deterministic models for interpretation of data are not yet available, and most data analysis is based on used of equivalent electrical circuits.

The objective of this work was to suggest a new method to analyze impedance data for skin without use of a detailed deterministic interpretation model.

### Experimental

Heat-stripped human cadaver skin was placed between two chambers of a diffusion cell joined by compression. Prior to the experiment, buffered electrolyte solution was added to each chamber of the cell. The system was maintained at 32 °C by a constant temperature water bath. Only the stratum corneum was used, as the literature indicates that it is the primary barrier to transport.[1] Skin samples were collected from the back and abdominal areas, and had little or no hair. The skin was visually examined before use to assure the integrity of the membrane. Experiments for which impedance scans found a polarization resistance approximately an order of magnitude below the normal range were discarded under the assumption that macroscopic shunt paths were present that were not detected during visual inspection.

Tests were conducted in buffered electrolyte solutions which contained either a monovalent or a divalent chloride salts. To insure that activity coefficient corrections were roughly equivalent for the experiments conducted in the two electrolytes, the electrolyte concentrations were chosen to yield the same ionic strength. The results from each type of solution were compared to determine effect of cation charge on the impedance response of skin. Ag/AgCl working and counter electrodes were used (In Vivo Metric). Ag/AgCl reference electrodes (Micro Electrodes Inc.) were placed on either side of the membrane, as close to the surfaces as possible, to yield the best estimates for trans-membrane potential and current measurements.

The electrochemical instrumentation consisted of a Solartron 1286 electrochemical interface and Solartron 1250 frequency response analyzer. Variable-amplitude galvanostatically-modulated impedance spectroscopy was employed in which the current amplitude was adjusted at each frequency to maintain the voltage response of the skin below a predetermined value.[2-4] This approach avoids inducing large potential gradients, which can alter skin properties.[5] Previously measured impedance values were used to predict the impedance at the frequency of the measurement being conducted. In-house software written for LabView® was used to control input parameters. The methods of Agarwal *et al*.[6,7] were used to ensure that the spectra used for the present analysis were consistent with the Kramers-Kronig relations.

### Results

The interpretation was based on use of the measurement model developed by Agarwal et al.[8] The interpretation followed two approaches.

<u>Analysis of Variance</u>

A large database of impedance measurements was collected during the course of this work. A statistical analysis of variance was performed for the polarization impedance and characteristic frequencies. Only the data shown to be consistent with the Kramers-Kronig relations were used for this analysis, and the polarization impedance was obtained by extrapolation of the impedance data using the measurement model.

The data displayed a wide distribution of impedance values. For example, the polarization resistance from pieces of skin extracted from adjacent sites on the same donor sample was distributed over several orders of magnitude. The variability of impedance and time constant between adjacent sites on the same donor sample was as large as found between different skin donors.

The distribution of the logarithm of skin impedance and characteristic frequency appeared to be bimodal.

<u>Distribution of Time Constants</u>

The depressed semicircle character of the impedance response of skin has been attributed to a distribution of relaxation time constants. This argument has been used to support use of a constant-phase-element model to interpret impedance data. Use of a constant-phase-element model is somewhat problematic because it requires an a-priori assumption of a distribution of relaxation time constants that is symmetric around a characteristic time constant. Regression of the measurement model was used here as a means to identify the distribution of time constants without a prior assumption of a distribution. This analysis shows that the distribution of relaxation time constants was symmetric during hydration of skin under zero-current conditions, but was greatly affected by passage of electrical current.

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